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Effects of asiaticoside on levels of podocyte cytoskeletal proteins and renal slit diaphragm proteins in adriamycin-induced rat nephropathy

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ABSTRACT

Aims: *Centella asiatica* has been used to treat kidney diseases in Chinese traditional medicine. Asiaticoside (an extraction of *C. asiatica*) exerts a variety of pharmacological effects including immunomodulatory and anti-inflammatory functions. However, the mechanism of asiaticoside in the treatment of renal diseases remains largely unknown. This study investigated the molecular mechanism of asiaticoside in treating adriamycin-induced nephropathy of rats.

Main methods: Sixty-two SD male rats were randomly divided into normal control group ($n = 12$) and nephropathy group ($n = 50$). Except for the normal control group, rats were injected with adriamycin (6 mg/kg) via the tail vein to induce nephropathy. Adriamycin induced nephropathic rats were divided into untreated group, prednisone group (25 mg/kg), and asiaticoside groups with various dosages (8, 16 and 32 mg/kg). Samples of urine and serum, tissue of kidney were collected for analysis after treatments for four weeks. Morphological changes were evaluated under light microscope and electron microscope. Synaptopodin, desmin, nephrin and podocin mRNA and protein were determined by RT-PCR and Western blotting.

Key findings: Compared to the untreated nephropathy group, asiaticoside treatment mitigated histological damages, decreased 24-hour urine protein excretion and total cholesterol, increased serum albumin. Asiaticoside treatment increased the mRNA and protein levels of synaptopodin, nephrin and podocin in a dose-dependent manner. Furthermore, asiaticoside treatment decreased the mRNA and protein levels of desmin.

Significance: Asiaticoside can mitigate adriamycin-induced nephropathy in rats, which is associated with the increase in synaptopodin, nephrin and podocin gene expression, and the decrease in desmin gene expression.

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Introduction

Proteinuria is one of the most common features of renal disease and is a consequence of problems with the glomerular filtration barrier. The glomerular filtration barrier is composed of capillary endothelial cells, glomerular basement membrane (GBM) and the slit diaphragm located between the foot processes of podocytes. Since the fenestrae of endothelial cells have large diameters, filtration of the protein by this portion of the barrier is limited. The GBM has a surface rich with anionic charges and functions as a charge barrier, and is composed of fibers arranged in a fine sieve-like network. Podocytes are located outside of the GBM and are divided structurally and functionally into three portions: cell body, major processes, and foot processes. Microtubules maintain and alter the morphology of podocyte foot process by arranging an actin-based

cytoskeletal system. Adjacent podocyte foot processes interdigitate, with interstices bridged by a slit diaphragm (SD). The SD filtration membrane barrier is the most important portion of the large filtration barrier (Ishizuka et al., 2007). Podocytes are an end-stage differentiated cell, lacking regenerative capacity following abscission (Kerjaschki, 2001). They comprise the last barrier against the protein loss from the body. Once podocytes are injured, proteinuria develops. The special structure of podocytes mainly depends on the expression of cytoskeletal system components and a number of specific proteins. These specific protein molecules and proteins of the slit diaphragm are important portions of the podocyte barrier, regulating normal renal function.

The adriamycin-induced nephropathy model was reported in the early 1980s and has been widely used in the field of kidney disease. Adriamycin is a quinone-containing anthracycline antibiotic and can be reduced to a semiquinone radical by metabolism in the kidney. The latter reacts with oxygen to produce reactive oxygen, inducing lipid peroxidation in the glomerular epithelial cells and destruction of the structure and function of the filtration membrane and ultimately proteinuria (Oka et al., 2008). An acute adriamycin-induced nephropathy model is induced by a single tail vein injection of 5–7.5 mg/kg adriamycin. This model similar to human minimal change nephrotic

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syndrome has been well characterized as an experimental model for nephrotic syndrome.

Centella asiatica is a member of the umbelliferae group of plants, and may reduce swelling, stimulate circulation, and promote diuresis (Qi and Wang, 2010). *C. asiatica* has been used to reduce urinary protein excretion in the treatment of kidney diseases for thousands of years in China. The chemical components of *C. asiatica* include triterpenoid saponins, triterpene acids, polyacetylene alkenes and volatile oils (Chen and Sun, 2006). Asiaticoside is widely used clinically for its pharmacological properties, such as immunomodulatory, anti-inflammatory, and antiviral properties, as well as promotion of wound healing (Yang, 2008). However, the exact mechanism of action of asiaticoside in the treatment of renal diseases remains largely unknown. The present study was designed to examine therapeutic roles of asiaticoside in adriamycin-induced nephropathy in rats. Specifically, effects of asiaticoside on expression of synaptopodin, desmin, nephrin and podocin were examined.

Materials and methods

Animals

Sixty-two male healthy SPF-grade SD rats (150 ± 20 g) were provided by the Experimental Animal Center of the School of Medicine, Xi'an Jiaotong University. Rats were housed under conditions of constant temperature (21 ± 2 °C) and humidity (60%) and exposed to a 12-h dark-light cycle. All rats were fed on a standard pellet diet and water was freely available.

Experimental design

Rats were randomly divided into a normal control group (Group A, n = 12) and a nephropathy model group (n = 50). The nephropathy model was prepared as described previously (Ren et al., 2010). Rats were injected with adriamycin (6 mg/kg, single dose) via the tail vein to induce nephropathy. The control group rats were injected with saline. Nephropathy was confirmed after three weeks by massive proteinuria, hypoalbuminemia, hyperlipidemia as well as by foot process effacement illustrated by electron microscopy (at the end of seventh week). Forty nephropathy rats were randomly divided into untreated nephropathy model group (Group B), asiaticoside groups (Groups C, D, and E, n = 8, respectively) and a positive control group (Group F, n = 8). Prednisone was used as a positive control (Gipson et al., 2009). From the 4th week on, Groups A and B were administered saline; Groups C, D, and E were administered 8 mg/kg, 16 mg/kg, and 32 mg/kg asiaticoside (Zhang et al., 2007), respectively; Group F was administered 25 mg/kg prednisone. All drugs and saline were administered by gavage once a day for 4 weeks.

At the end of the 3 and 7 weeks after the first adriamycin injection, 24 h urine samples were collected by metabolic cages; blood was collected from the abdominal aorta, and serum was prepared via centrifugation and stored at −20 °C. At the end of the 7 weeks, all rats were euthanized. Parts of fresh kidney tissues were removed and fixed in 10% formaldehyde for routine histological examination; whereas another part of the kidney tissues was fixed in 2% glutaraldehyde for transmission electron microscopy examination. The remaining kidney tissues were kept in liquid nitrogen for protein and mRNA measurements. All animal procedures were conducted in accordance with the China Animal Welfare Legislation and were approved by the Ethics Committee on the Care and Use of Laboratory Animals in Xi'an Jiaotong University (Shaanxi, China).

Biochemical analyses

Twenty four-hour urine protein excretion was measured by pyrogallol red-molybdate method (DiaSys Diagnostic Systems Co., Ltd, Shanghai). The levels of albumin (ALB), serum triglyceride (TG), total

cholesterol (TC), urea nitrogen (BUN), and creatinine (Cr) were detected by Automatic Analyzer (7600-020, Hitachi, Tokyo, Japan).

Histological examination

The fixed tissue specimens were dehydrated in graded alcohol solutions, cleared in toluene, and embedded in paraffin. Sections (3 μm) were stained with periodic acid-Schiff stain (PAS). For the electron microscopic analysis, 1 × 1 × 1 mm³ fragments of the renal tissues were fixed overnight in cold glutaraldehyde (2%) in 0.1 M sodium cacodylate buffer for 2 h in 4 °C. After dehydration in graded ethanol, the pellets were embedded in Spurr. Ultra-thin sections were observed with the electron microscope (H600A, Hitachi, Tokyo, Japan).

Real-time-polymerase chain reaction (PCR)

Total RNA was purified from fresh rat kidney cortex tissue (50–100 mg) on ice with RNeasy1000 (Pioneer, China). The purity of total RNA was checked with a spectrophotometer and the wavelength absorption ratio (260/280 nm) was between 1.7 and 2.0 in all preparations. Reverse transcription of total RNA (0.1–5 μg) to cDNA was carried out using RevertAid First Strand cDNA Synthesis Kits (MBI, Lithuania) in 20 μL volume reaction at 44 °C for 1 h by using Mastercycler personal PCR machine (Eppendorf AG, Hamburg, Germany). Specific primers were designed using Beacon Designer 4.0 software and synthesized with Sunbiotech (Beijing Sunbiotech CO., Ltd, China). Real-time PCR was performed with Maxima SYBR Green/ROX qPCR Master Mix (2×) (MBI, Lithuania) in the IQ5.0 system (BioRad, USA). The system automatically monitors the binding of a fluorescent dye SYBR® Green to double-stranded DNA during each cycle of PCR amplification. PCR was performed under the following conditions: Heating 50 °C for 2 min, followed by 95 °C for 10 min and 40 PCR cycles with 94 °C for 15 s, 60 °C for 30 s, 72 °C for 30 s. The relative amount of mRNA was calculated using the comparative C_t (ΔΔC_t) equation. The β-actin gene was used as a reference for normalizing data. Derived normalized values are the mean of three runs. The sequences of the rat and human primers (Beijing Sanbo Yuanzhi Biotechnology, Ltd) are listed in Table 1.

Western blotting

Kidney tissue homogenates or cells were lysed by RIPA (radioimmuno-precipitation assay) lysis buffer (Pioneer, China), which enables the extraction of cytoplasmic, membrane and nuclear proteins. Samples containing 100 μg of protein were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a PVDF membrane. Nonspecific binding was blocked with 5% non-fat dry milk in 0.1% Tween-20/TBS (TBST) for 1 h at room temperature. Following washing with TBST, the blots were incubated overnight at 4 °C with the primary antibody: synaptopodin, 1:500 (Proteintech, USA), desmin, 1:1000 (Proteintech, USA), podocin, 1:200 (Proteintech, USA), nephrin, 1:200 (Abcam, USA), and β-actin, 1:2000 (Santa Cruz Biotechnology, USA). The membranes were incubated for 1 h at room temperature with the corresponding HRP-conjugated secondary antibodies (synaptopodin, desmin, podocin, and nephrin were goat anti-rabbit IgG(H + L); β-actin was goat anti-mouse IgG(H + L), Pierce, USA). Signals were visualized using a GE

Table 1
Real-time PCR primer sequences.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
Synaptopodin	CCACAGAGGCACATAATG	GGATACAGACTAGAATAAGAGG
Desmin	TCCTACACCTGCGAGATTG	GCGATGTTGCTCTGATAGC
Nephrin	TTCTGCTGCTCTCCAATG	ACTTCTGCTGTGCTAACC
Podocin	GGTGATTGCTGCCGAAGG	CTGTGACAAAGGACTGAAGAG

Table 2

Twenty four hours urinary protein excretion at the end of 3rd week.

Groups	n	24 h urinary protein excretion (mg/24 h)
A	12	37.99 ± 14.99
B–F	40	109.82 ± 25.67#

(A) Normal control group. (B) Nephropathy model group. (C) Asiaticoside (8 mg/kg) group. (D) Asiaticoside (16 mg/kg) group. (E) Asiaticoside (32 mg/kg) group. (F) Prednisone (25 mg/kg) group. Values were expressed as mean ± S.D. #, $P < 0.05$ vs. Group A.

Table 3

Twenty four hours urinary protein excretion at the end of 7th week.

Groups	n	24 h urinary protein excretion (mg/24 h)
A	12	42.32 ± 10.15
B	8	162.77 ± 27.51#
C	8	140.08 ± 15.23▲
D	8	109.77 ± 19.64▲
E	8	107.90 ± 8.94▲
F	8	97.55 ± 12.82▲

(A) Normal control group. (B) Nephropathy model group. (C) Asiaticoside (8 mg/kg) group. (D) Asiaticoside (16 mg/kg) group. (E) Asiaticoside (32 mg/kg) group. (F) Prednisone (25 mg/kg) group. Values were expressed as mean ± S.D. #, $P < 0.05$ vs. Group A; ▲, $P < 0.05$ vs. Group B.

ImageQuant LAS4000mini, quantified using densitometry with the Gel Doc XR System (Bio-Rad Laboratories, Hercules, CA, USA), and analyzed using Quantity One Protein Analysis Software (Bio-Rad Laboratories, Hercules, CA, USA).

Reagents

Asiaticoside with purity over 95% was purchased from Guangxi Changzhou Natural Products Development Co., Ltd, China (Batch number: 110124). Asiaticoside was dissolved in 0.5% sodium carboxymethyl cellulose. Adriamycin was provided by Zhejiang Haizheng Pharmaceutical Co., Ltd., China. Prednisone acetate was obtained from Xi'an Lijun Pharmaceutical Co., Ltd., China.

Statistical analyses

SPSS version 16.0 for Windows was used for all analyses. All values are expressed as mean ± SD. Statistical significance between experimental groups was determined by a *t* test or analysis of variance (ANOVA). P values < 0.05 were considered statistically significant.

Results

Determination of 24 h urinary protein excretion

As indicated above, forty nephropathy rats were randomly divided into untreated nephropathy model group, asiaticoside groups with variable dosages (8 mg/kg, 16 mg/kg and 32 mg/kg) and a positive control group. As shown in Tables 2 and 3, after 3 weeks of adriamycin injection, 24 h urinary protein excretion increased in rats with Group B compared with rats in Group A ($P < 0.05$), suggesting that the nephropathy model was successfully established. At the end of the

experiment, urinary protein excretion was significantly reduced in the asiaticoside-treated groups (Group C, D, and E) and the positive control group (Group F) ($P < 0.05$).

Biochemical analyses

Serum ALB in Group B decreased three weeks following adriamycin injection, while serums TC and TG were significantly higher than those in Group A ($P < 0.05$) (Table 4). At the end of the seventh week, ALB in Groups C, D, and E were higher than that in Group B. In addition, as shown in Table 5, asiaticoside treatment reduced TC and TG compared with Group B ($P < 0.05$).

Histological findings

As shown in Fig. 1A, under the light microscopic examination, no evident pathological changes were found in the kidney of Group a. Whereas in Group b, the glomerular changes consisted of proliferation of glomerular mesangial cells, increase of mesangial matrix and glomerular capillary lumen narrowing; the tubular changes were characterized by inflammatory cell infiltration in the renal interstitium. In Groups d, e and f, these pathological damages (indicated with arrows in Fig. 1A) were lighter than those of Group b, especially in Group e.

As shown in Fig. 1B, under the electron microscope, integrity of podocyte structure, clear foot process, and intact basal structure were observed in the kidney of Group a. In Group b, foot processes were broadened, fused, evenly disappeared, along with podocyte loss and cell number reduction. There was no apparent improvement in pathological damages in Group c; whereas in Groups d, e and f, the severity of pathological damages (indicated with arrows in Fig. 1B) was clearly ameliorated, especially identified by the recovery of the structure and the number of podocyte foot process. These improvements were mostly observed in Group e.

mRNA levels

As shown in Fig. 2, mRNA levels of synaptopodin, nephrin and podocin in adriamycin-induced nephropathy model were significantly decreased, whereas the mRNA expression of desmin was increased when compared with the normal control group ($P < 0.05$). In contrast, asiaticoside treatment increased the mRNA levels of synaptopodin, nephrin and podocin, and decreased the levels of desmin mRNA in a dose-dependent manner ($P < 0.05$).

Protein levels

As shown in Fig. 3, protein levels of synaptopodin, nephrin and podocin were significantly decreased, whereas desmin protein was increased in adriamycin-induced nephropathy model compared with the control group ($P < 0.05$). In contrast, asiaticoside treatment increased the protein levels of synaptopodin, nephrin and podocin, and decreased the levels of desmin protein in a dose-dependent manner ($P < 0.05$).

Table 4

Biochemical parameters at the end of 3rd week after the first adriamycin injection.

Groups	n	ALB (g/L)	BUN (mmol/L)	Cr (μmol/L)	TC (mmol/L)	TG (mmol/L)
A	12	31.69 ± 1.21	8.31 ± 0.65	49.65 ± 3.22	1.54 ± 0.44	1.35 ± 1.15
B–F	40	27.59 ± 2.77#	10.78 ± 2.12	57.44 ± 5.93	11.41 ± 0.96#	9.24 ± 5.4#

(A) Normal control group. (B) Nephropathy model group. (C) Asiaticoside (8 mg/kg) group. (D) Asiaticoside (16 mg/kg) group. (E) Asiaticoside (32 mg/kg) group. (F) Prednisone (25 mg/kg) group. Values were expressed as mean ± S.D. #, $P < 0.05$ vs. Group A.

Table 5

Biochemical parameters at the end of 7th week after the first adriamycin injection.

Groups	n	ALB (g/L)	BUN (mmol/L)	Cr (μ mol/L)	TC (mmol/L)	TG (mmol/L)
A	12	30.24 \pm 1.38	7.89 \pm 1.57	53.80 \pm 5.62	1.79 \pm 0.34	1.52 \pm 0.52
B	8	20.85 \pm 2.08#	10.52 \pm 3.62	57.10 \pm 8.13	12.89 \pm 1.59#	10.76 \pm 2.05#
C	8	22.93 \pm 4.75	11.78 \pm 2.56	57.48 \pm 5.44	10.47 \pm 2.30	8.72 \pm 3.84
D	8	25.93 \pm 3.18▲	11.48 \pm 3.77	55.84 \pm 5.20	9.83 \pm 1.96	5.36 \pm 2.13▲
E	8	26.13 \pm 1.49▲	9.05 \pm 1.82	54.85 \pm 4.07	8.79 \pm 1.73▲	5.07 \pm 2.72▲
F	8	30.55 \pm 6.3▲	9.67 \pm 5.99	57.08 \pm 8.58	8.48 \pm 1.41▲	18.84 \pm 7.13

(A) Normal control group. (B) Nephropathy model group. (C) Asiaticoside (8 mg/kg) groups. (D) Asiaticoside (16 mg/kg) group. (E) Asiaticoside (32 mg/kg) group. (F) Prednisone (25 mg/kg) group. Values were expressed as mean \pm S.D. #, $P < 0.05$ vs. Group A; ▲, $P < 0.05$ vs. Group B.

Discussion

Podocytes are located at the outer layer of GBM and possess foot processes that interdigitate and create small pores that are spanned by SD; podocytes and SDs are the last component of the glomerular filtration barrier. SD has been observed via electron microscopy to resemble a zipper-like structure spanning the space between foot processes (Rodewald and Karnovsky, 1974). The specialized structure

of a podocyte regulates its overall functions, which can be summarized as follows: (1) molecular protein filtration barrier; (2) protein filtration charge barrier; (3) resisting the hydrostatic pressure of the glomeruli; (4) maintaining the spatial structure of the glomerular capillary loops; (5) synthesizing the ingredients necessary for maintaining the integrity of GBM; and (6) synthesizing and secreting vascular endothelial growth factor to maintain the functional integrity of the glomerular endothelial cells (Shankland, 2006).

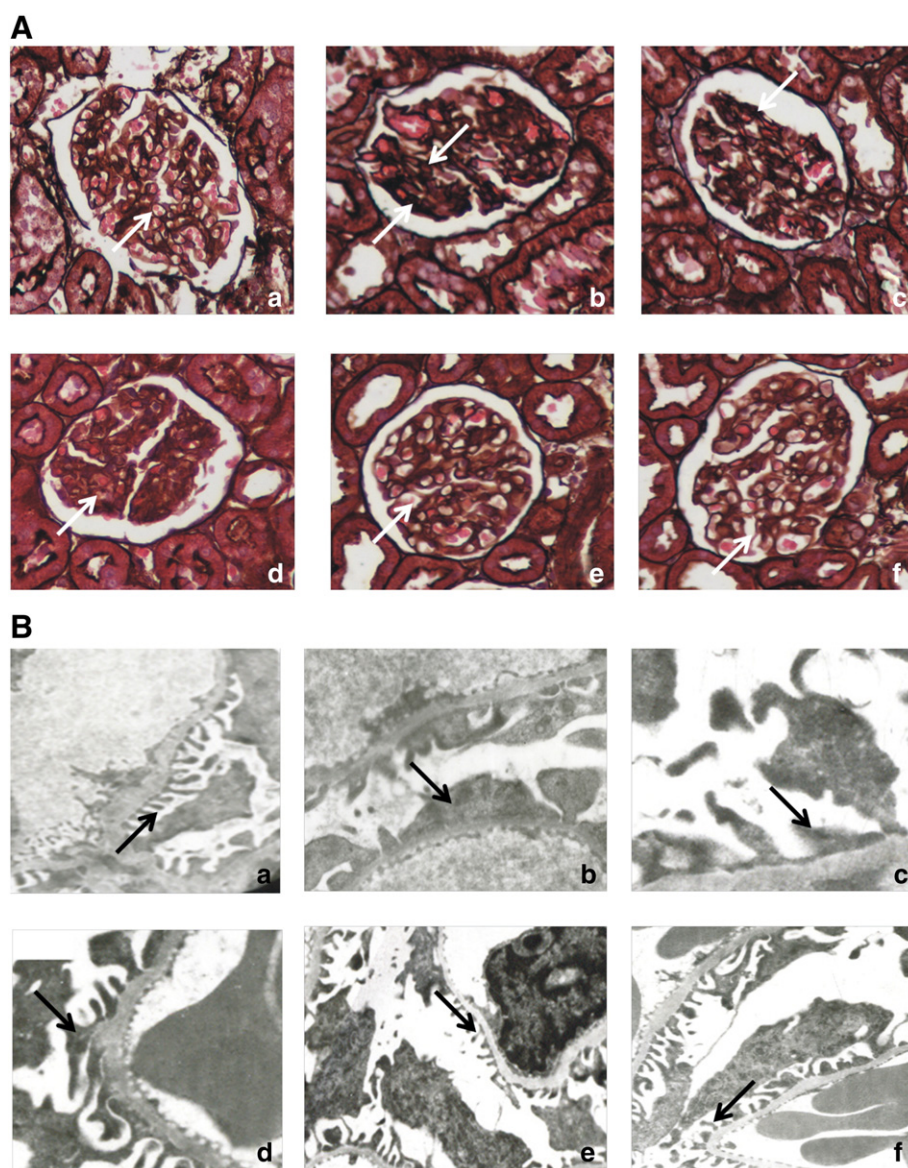


Fig. 1. Morphological changes of kidney after asiaticoside treatment demonstrated by light microscopy (PAS \times 400) (A) and transmission electron microscopy (\times 10,000) (B) of the kidneys in different groups. Panels represent images of tissues from animals with different treatments: (a) Normal control. (b) Nephropathy model. (c) Asiaticoside (8 mg/kg). (d) Asiaticoside (16 mg/kg). (e) Asiaticoside (32 mg/kg). (f) Prednisone (25 mg/kg). Arrows indicate the structural changes (A) and foot processes (B) of the glomerulus.

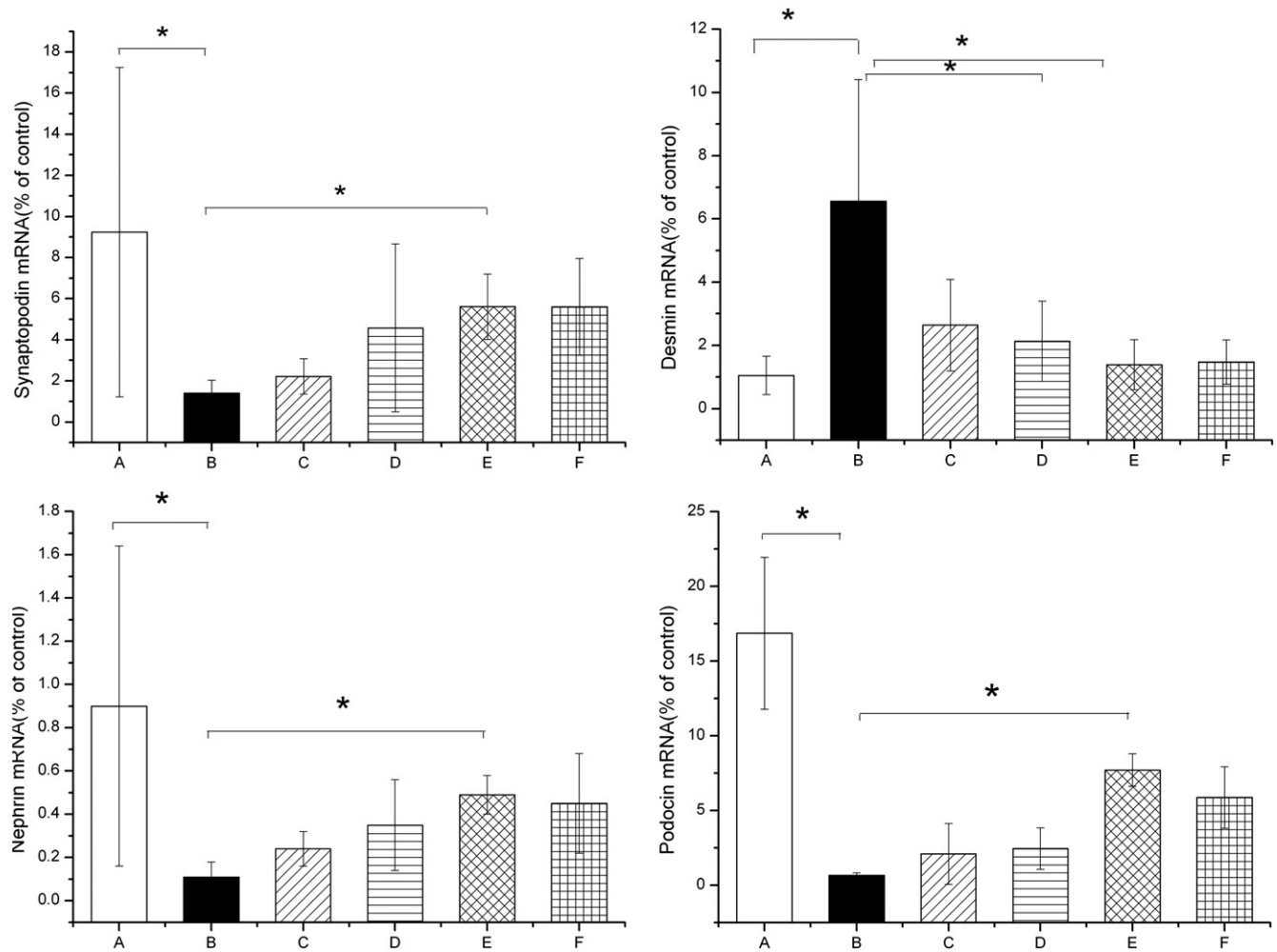


Fig. 2. Effects of asiaticoside on mRNA levels of synaptopodin, nephrin, desmin and podocin. Real-time PCR was performed using total RNA from kidney tissues. (A) Normal control group. (B) Nephropathy model group. (C) Asiaticoside (8 mg/kg) group. (D) Asiaticoside (16 mg/kg) group. (E) Asiaticoside (32 mg/kg) group. (F) Prednisone (25 mg/kg) group. The results of synaptopodin, nephrin, desmin and podocin levels were expressed as relative to the corresponding β -actin. Values were expressed as mean \pm S.D. ($n = 6$). *, $P < 0.05$ vs. Group B.

Synaptopodin and desmin are cytoskeletal proteins. During kidney development, synaptopodin is not expressed in the predecessor cells of podocytes (Sun and Wang, 2004). Therefore, synaptopodin is considered as an important marker of mature podocytes. Compared to normal kidney tissue, synaptopodin expression progressively declines in minimal change disorder (MCD) and mesangial proliferative glomerulonephritis (MSPGN). Furthermore, synaptopodin in tissue from kidneys with focal segmental glomerulosclerosis (FSGS) decreases markedly (Srivastava et al., 2001). The expression of synaptopodin is increased in patients with idiopathic collapsing glomerulopathy and HIV-associated nephropathy (Yang et al., 2002). Desmin is an intermediate filament protein of the cytoskeleton and is secreted by podocytes during injury (Floege et al., 1992). Desmin expression and podocyte injury are significantly correlated (Kerjaschki and Neale, 1996).

Nephrin and podocin are protein components of the filtration slit diaphragm. Nephrin expression is found only in the pores between glomerular podocytes (Miner, 1999). Thus, it is believed that nephrin is one of the main components of SD, a component of the renal filtration barrier. A gene mutation in NPHS1 can lead to the loss of nephrin expression, causing destruction of SD and significant proteinuria (Aoyama et al., 2006; Li et al., 2004). Reduced mRNA and protein expression of nephrin occurs earlier than ultrastructural changes in podocytes. Therefore, nephrin can be used as an early important biomarker for acute glomerular podocyte injury (Bo et al., 2003; Kucukardali et al., 2008). As a scaffolding protein, podocin plays an

important role in maintaining the structure and function of SD. In vitro and in vivo studies have confirmed that podocin plays a scaffolding role in lipid rafts of foot process membrane in podocytes, which are critical to the structure and function of SD (Browman et al., 2007; Philippe et al., 2008). Podocin mutant mice have early proteinuria and progressive renal failure, with pathological manifestations including severe mesangial sclerosis, wide fusion of foot processes, and SD disappearance (Philippe et al., 2008). Reduced expression of podocin is an initiating factor in the development of proteinuria (Liu et al., 2012). In summary, cytoskeletal proteins and SD proteins affect the structure and the function of podocytes.

Prevention and treatment of podocyte injury and amelioration of proteinuria are important interventional strategies for nephropathy. In the present study, histological changes (proliferation of glomerular mesangial cells, increased mesangial matrix, glomerular capillary lumen narrowing, and inflammatory cell infiltration in the renal interstitium) were shown under the light microscopic examination. Images under transmission electron microscope further demonstrated foot process effacement in the nephropathy group, indicating the success of model establishment. After the treatment with asiaticoside, the histological damages were mitigated, especially in the high dose of asiaticoside group. Furthermore, asiaticoside was able to reduce urine protein excretion in the adriamycin-induced nephropathy rats. Elevated serum albumin and lower total cholesterol content were also observed in asiaticoside treatment groups.

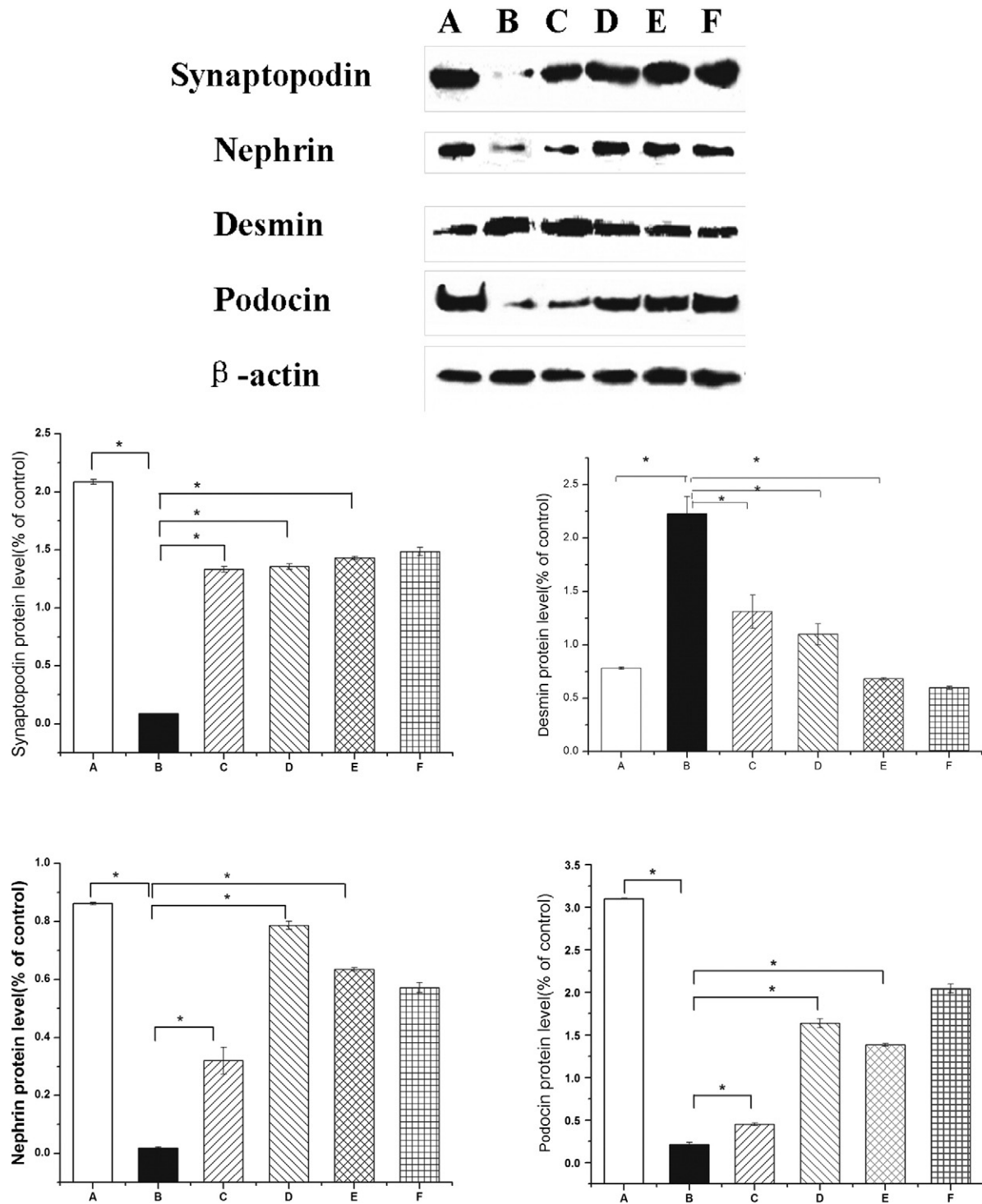


Fig. 3. Effects of asiaticoside on protein levels of synaptopodin, nephrin, desmin and podocin. Western blotting was performed using total protein from kidney tissues. (A) Normal control group. (B) Nephropathy model group. (C) Asiaticoside (8 mg/kg) groups. (D) Asiaticoside (16 mg/kg) group. (E) Asiaticoside (32 mg/kg) group. (F) Prednisone (25 mg/kg) groups. The results of synaptopodin, nephrin, desmin and podocin levels were expressed as relative to the corresponding β -actin. Values were expressed as mean \pm S.D. ($n = 6$). *, $P < 0.05$ vs. Group B.

In addition, asiaticoside increased the expression of synaptopodin and decreased desmin expression in rats with adriamycin-induced nephropathy, suggesting that asiaticoside restored the expression of cytoskeletal proteins in damaged podocytes. For SD proteins, asiaticoside increased the expression of nephrin and podocin in a dose-dependent manner. Taken together, our study suggests that asiaticoside protection against renal disease might be accomplished through altering foot process cytoskeleton and SD protein component expression, resulting in phenotypic changes in podocytes and subsequently restoring normal morphology and repair of the glomerular filtration barrier.

Asiaticoside is the main chemical extracted from *C. asiatica*. Our study provides insights into the mechanisms involved in *C. asiatica* treatment for kidney disease, and highlights new potential therapeutic targets for nephropathy. Although we have demonstrated that the alternative expressions of synaptopodin, nephrin, podocin and desmin genes are associated with the podocyte injury, their roles in the occurrence and development of proteinuria remain unclear. Additional mechanisms underlying the therapeutic effect of asiaticoside on podocytes in kidney disease require further studies.

Conclusion

In the present study, asiaticoside mitigated the histological damages, decreased 24-hour urine protein excretion, TC, and increased ALB in rats of adriamycin-induced nephropathy. These protective effects were associated with the increase in synaptopodin, nephrin and podocin gene expression, and the decrease in desmin gene expression.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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